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## One-dimensional SDS-PAGE (9-18% TGX gel)

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We use this protocol and it's working

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## Abstract

Protocol for one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 9-18% TGX gel for visualization and quantification of histone proteins.

## Troubleshooting



## Sample preparation

- 1 Dry samples (equal to 400.000 cells)
- 2 Resuspend samples in 10 $\mu$ l laemmli-buffer
- 3 Add 1 $\mu$ l  $\beta$ -mercaptoethanol to each sample

### Safety information

Perform this step in a fume hood

- 4 Vortex and spin down
- 5 Incubate for 7 minutes at 95°C in a thermoshaker
- 6 Spin down

## Prepare Criterion Cell

- 7 Place the criterion cell on ice in a fume hood
- 8 Remove the sticker from the bottom of the gel cassette and check the gel for cracks
- 9 Put the gel cassette in the criterion cell
- 10 Fill the reservoir with running buffer (25mM Tris, 0.1% SDS, and 192mM glycine in MilliQ water) and take out the comb




## Running of the samples

- 11 Load the samples and standards (2  $\mu$ g of bovine histones) on the gel (3 standards per gel: lane 1, lane 9 and lane 18)
- 12 Put the cover on the criterion cell
- 13 Start running the gel on 200V
- 14 Stop running when the frontline is almost gone



## Visualization

- 15 Take out the cassette
- 16 Incubate in fixation-solution (7% acetic acid, 10% methanol in MilliQ water) for 10 minutes on a shaker
- 17 Wash the gel 3 times for 5 minutes in MilliQ water on a shaker
- 18 Incubate in SyproRuby overnight  
 Overnight
- 19 Wash the gel 3x for 10 minutes in MilliQ water on a shaker
- 20 Visualize the gel (Versadoc)